Criteria Specification

ClinGen Cerebral Creatine Deficiency Syndromes Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for GATM Version 1.1.0

Affiliation: Cerebral Creatine Deficiency Syndromes VCEP
Description : Cerebral Creatine Deficiency Syndromes Variant Curation Expert Panel ACMG Classification Rules Specified for GATM (arginine:glycine amidinotransferase) Summary of ACMG-AMP Criteria for GATM Variants
Version : 1.1.0
Released : 9/14/2022
Release Notes :

- Added supporting strength for PM3 and PM4. This was previously approved by SVI for Version 1.0 but had not been included in the CSpec Registry.
- Added clarification for points system for PP4, all previously approved by SVI.

Rules for GATM

Gene: GATM (HGNC:4175)
Preferred Transcript: NM_001482.3
HGNC Name: glycine amidinotransferase
Disease: AGAT deficiency (MONDO:0012996)

Criteria & Strength Specifications

PVS1

Original ACMG Summary

Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.
Caveats:
• Beware of genes where LOF is not a known disease mechanism (e.g. GFAP, MYH7).
• Use caution interpreting LOF variants at the extreme 3’ end of a gene.
• Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact.
• Use caution in the presence of multiple transcripts.

Very Strong

Nonsense-mediated decay predicted.

CCDS VCEP notes:
Loss of function (LOF) of GATM is a known mechanism of disease for arginine:glycine amidinotransferase deficiency (AGAT-D). There are examples of various LOF variants, including nonsense and frameshift, in GATM in individuals with AGAT-D

(https://databases.lovd.nl/shared/variants/GATM/unique). The specifications below are based on the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042).

GATM specifications:

Nonsense and frameshift variants

* All nonsense and frameshift variants will meet PVS1 unless a premature termination codon is predicted to be missed by nonsense-mediated decay (NMD) because it is located in the last exon (exon 9) or the last 50 bases of the penultimate exon (exon 8, 3' of c.1109). In that case, PVS1_Strong or PVS1_Moderate will be applied depending on whether >10% or <10% of the protein is lost.

Splice site variants (+1, +2, -1, -2)

* All canonical splice site pairs in GATM are GT-AG.
* For any canonical splice site variant (+1, +2, -1, -2), the exon immediately adjacent to the variant is predicted to be skipped i.e. upstream exon skipped for canonical donor splice site variants and downstream exon skipped for canonical acceptor splice site variants.
* For the predicted in frame/out of frame consequences of exon skipping and considerations for strength of PVS1, see Appendix 1.
* If this criterion is applied, PP3 (in silico splice site prediction tools) should not be used.
* To apply PVS1, splice site variants must have no detectable nearby (+/- 20 nucleotides) strong consensus splice sequence that may reconstitute in-frame splicing. Otherwise, the PVS1 strength should be reduced accordingly.
* Non-canonical splice variants, such as +3 or -3, will not meet PVS1, but could meet PS3 and/or PP3 criteria.

Deletions (single or multi exon)

* If a single or multi-exon deletion results in an out of frame consequence, use PVS1 unless not predicted to undergo NMD. If not predicted to undergo NMD, use PVS1_Strong if >10% of the protein is predicted to be removed, and use PVS1_Moderate if <10% of the protein is predicted to be removed.
* If the consequence is in frame, the deletion must encompass one or more exons for PVS1 to apply. Use PVS1_Strong if more than 10% of the protein is removed and PVS1_Moderate if <10% of the protein is removed.
* If the in frame deletion is smaller than one exon, PVS1 does not apply; consider using PM4.
* Appendix 1 can be used to predict the consequences of single exon deletions.

Duplications

* Use the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042) to assess the impact of duplications.

Modification Type: None

Strong

In frame loss of >10% of the protein.

CCDS VCEP notes:

Loss of function (LOF) of GATM is a known mechanism of disease for arginine:glycine amidinotransferase deficiency (AGAT-D). There are examples of various LOF variants, including nonsense and frameshift, in GATM in individuals with AGAT-D (https://databases.lovd.nl/shared/variants/GATM/unique). The specifications below are based on the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042).

GATM specifications:

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Splice site variants (+1, +2, -1, -2)

* All canonical splice site pairs in GATM are GT-AG.
* For any canonical splice site variant (+1, +2, -1, -2), the exon immediately adjacent to the variant is predicted to be skipped i.e. upstream exon skipped for canonical donor splice site variants and downstream exon skipped for canonical acceptor splice site variants.
*Use SpliceAI and varSEAK to look for nearby (+/- 20 nucleotides) strong consensus splice sequence that may reconstitute in-frame splicing.
* For considerations for strength at which PVS1 may be applied see Appendix 1.
* If this criterion is applied, PP3 (in silico splice site prediction tools) should not be used.
* Non-canonical splice variants, such as +3 or -3, will not meet PVS1, but could meet PS3 and/or PP3 criteria.

Deletions (single or multi exon)

* If a single or multi-exon deletion results in an out of frame consequence, use PVS1 unless not predicted to undergo NMD. If not predicted to undergo NMD, use PVS1_Strong if >10% of the protein is predicted to be removed, and use PVS1_Moderate if <10% of the protein is predicted to be removed.
* If the consequence is in frame, the deletion must encompass one or more exons for PVS1 to apply. Use PVS1_Strong if more than 10% of the protein is removed and PVS1_Moderate if <10% of the protein is removed.
* If the in frame deletion is smaller than one exon, PVS1 does not apply; consider using PM4.
* Appendix 1 can be used to predict the consequences of single exon deletions.

Duplications

* Use the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042) to assess the impact of duplications.

Modification Type: Strength

Moderate

Single exon or larger deletion resulting in loss of <10% of the protein, and

initiator codon variants.

CCDS VCEP notes:
Loss of function (LOF) of GATM is a known mechanism of disease for arginine:glycine amidinotransferase deficiency (AGAT-D). There are examples of various LOF variants, including nonsense and frameshift, in GATM in individuals with AGAT-D (https://databases.lovd.nl/shared/variants/GATM/unique). The specifications below are based on the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042).

GATM specifications:

Nonsense and frameshift variants
* All nonsense and frameshift variants will meet PVS1 unless a premature termination codon is predicted to be missed by nonsense-mediated decay (NMD) because it is located in the last exon (exon 9) or the last 50 bases of the penultimate exon (exon 8, 3' of c.1109). In that case, PVS1_Moderate will be applied.

Splice site variants (+1, +2, -1, -2)
* All canonical splice site pairs in GATM are GT-AG.
* For any canonical splice site variant (+1, +2, -1, -2), the exon immediately adjacent to the variant is predicted to be skipped i.e. upstream exon skipped for canonical donor splice site variants and downstream exon skipped for canonical acceptor splice site variants.
*Use SpliceAI and varSEAK to look for nearby (+/- 20 nucleotides) strong consensus splice sequence that may reconstitute in-frame splicing.
* For considerations for strength at which PVS1 may be applied see Appendix 1.
* If this criterion is applied, PP3 (in silico splice site prediction tools) should not be used.
* Non-canonical splice variants, such as +3 or -3, will not meet PVS1, but could meet PS3 and/or PP3 criteria.

Initiator codon variants
* To our knowledge, initiator codon variants have not been reported in GATM (01/2019) but may occur.
* All initiator codon variants will meet PVS1_Moderate. The next in-frame methionine is at amino acid position 130 (based on NP_001473).

Deletions (single or multi exon)
* If a single or multi-exon deletion results in an out of frame consequence, use PVS1 unless not predicted to undergo NMD. If not predicted to undergo NMD, use PVS1_Strong if >10% of the protein is predicted to be removed, and use PVS1_Moderate if <10% of the protein is predicted to be removed.
* If the consequence is in frame, the deletion must encompass one or more exons for PVS1 to apply. Use PVS1_Strong if more than 10% of the protein is removed and PVS1_Moderate if <10% of the protein is removed.
* If the in frame deletion is smaller than one exon, PVS1 does not apply; consider using PM4.
* Appendix 1 can be used to predict the consequences of single exon deletions.

Duplications
* Use the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042) to

assess the impact of duplications.

Modification Type: Strength

PS1

Original ACMG Summary

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.
Example: Val->Leu caused by either G>C or G>T in the same codon.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Strong

- This criterion is applicable as described.
- If the variant is in the last 3 nucleotides of an exon, further analysis using splicing site prediction algorithms (see PP3) and data from the literature (if available) is required to investigate the impact on splicing.

Modification Type: None

PS2

Original ACMG Summary

De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.
Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity.

Not Applicable

Comments: De novo (both maternity and paternity confirmed) in a patient with the disease and no family history. Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to non-maternity. CCDS VCEP notes for PS2 and PM6: De novo variants have not been reported in patients with AGAT deficiency, to our knowledge. Furthermore, the observation that a variant in GATM has arisen de novo does not support its causality because AGAT deficiency is an autosomal recessive disorder. The occurrence of de novo variants is more supportive in autosomal dominant and X-linked disorders. Any de novo variants in GATM, should they be observed, will be

assessed based on the variant type, functional evidence, and in trans data as described.

PS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.
Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established.

Strong

RT-PCR evidence of mis-splicing for non-canonical intronic variants with no evidence of normal splice products.

GATM specifications:
Any variant meeting the description for either in vitro expression or splicing assays can meet PS3 at the strengths given. If a variant meets the description for both e.g., a splice site variant with no evidence of abnormal splicing and deficient AGAT activity in vitro, PS3 must only be applied once.

Splicing assays
For non-canonical splicing variants, use PS3 if there is RT-PCR and/or RNA sequencing evidence demonstrating only abnormal splice products, with no evidence of normal splicing.
* Evidence of abnormal splicing includes transcripts of alternative length or with specific intron or exon inclusion/exclusion. These studies can be performed on mRNA extracted from patient-derived cells, or by inserting the mutant genomic DNA into plasmid vectors and introducing these into human or other mammalian host cells.
* Note that in patients who are compound heterozygotes for a splicing variant and another variants type that does not disrupt splicing, such as a missense variant, evidence of normal splicing is expected. However, the presence of normal splice products could complicate the assessment of the impact of the splice variant. Therefore, if there is any evidence of normal splice products, either when using RNA from patient cells or in an in vitro expression system, use PS3_Supporting.
* PP3 may also be used for non-canonical splice variants meeting PS3 or PS3_Supporting.

Modification Type: Disease-specific

Supporting

<15% control activity when variant is expressed in HeLa cells, as reported in PMID 27233232.
RT-PCR evidence of mis-splicing for non-canonical intronic variants with evidence of normal splice products.

GATM specifications:
Any variant meeting the description for either in vitro expression or splicing assays can meet PS3 at the strengths given. If a variant meets the description for both e.g., a splice site variant with no evidence of abnormal splicing and deficient AGAT activity in vitro, PS3 must only be applied once.

In vitro expression
AGAT activity data from an in vitro assay in which GATM variants were overexpressed in HeLa cells has been published (DesRoches et al, 2016; PMID 27233232). Any variant with AGAT activity at or below 15% of normal in this paper meets PS3_Supporting (see Appendix 2 for further details on AGAT functional assays).

Splicing assays
For non-canonical splicing variants, use PS3 if there is RT-PCR and/or RNA sequencing evidence demonstrating only abnormal splice products, with no evidence of normal splicing.
* Evidence of abnormal splicing includes transcripts of alternative length or with specific intron or exon inclusion/exclusion. These studies can be performed on mRNA extracted from patient-derived cells, or by inserting the mutant genomic DNA into plasmid vectors and introducing these into human or other mammalian host cells.
* Note that in patients who are compound heterozygotes for a splicing variant and another variants type that does not disrupt splicing, such as a missense variant, evidence of normal splicing is expected. However, the presence of normal splice products could complicate the assessment of the impact of the splice variant. Therefore, if there is any evidence of normal splice products, either when using RNA from patient cells or in an in vitro expression system, use PS3_Supporting.
* PP3 may also be used for non-canonical splice variants meeting PS3 or PS3_Supporting.

Modification Type: Disease-specific

PS4

Original ACMG Summary

The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.
Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0. See manuscript for detailed guidance.
Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Not Applicable
Comments: CCDS VCEP notes for PS4: This rule is typically used for autosomal

dominant disorders, with PM3 used as a case-counting mechanism for autosomal recessive conditions.

PM1

Original ACMG Summary

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

Not Applicable

Comments: Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation.

PM2

Original ACMG Summary

Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.
Caveat: Population data for indels may be poorly called by next generation sequencing.

Supporting

Allele frequency <0.000055 (<0.0055%) in all populations in gnomAD.

CCDS VCEP notes: It is acceptable for a GATM variant to be present in controls, if heterozygous, because AGAT-D is a recessive disorder. Homozygotes should not be seen in a population database, such as gnomAD, because the penetrance of this condition in individuals with biallelic pathogenic variants is expected to be 100%.

GATM specifications:

- All subpopulations in gnomAD must have a maximum allele frequency less than 0.000055 (based on the prevalence of the most common suspected pathogenic variants, c.484+1G>T and p.Arg169Ter) (see Appendix 3). Note – PM2 will NOT be used at moderate strength; PM2 will only be applied as a Supporting criterion.
- If homozygotes are observed, the variant will meet BS2 (assuming 100% penetrance for an individual with 2 pathogenic variants in trans).

Modification Type: Disease-specific,Strength

PM3

Original ACMG Summary

For recessive disorders, detected in trans with a pathogenic variant
Note: This requires testing of parents (or offspring) to determine phase.

Very Strong

- Follow SVI guidance for PM3 (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf).
- Parental testing, or another appropriate molecular method (such as cloning each allele separately followed by sequencing), must have been performed in order to confirm that the variants are in trans if the patient is compound heterozygous.

Modification Type: Strength

Strong

- Follow SVI guidance for PM3 (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf).
- Parental testing, or another appropriate molecular method (such as cloning each allele separately followed by sequencing), must have been performed in order to confirm that the variants are in trans if the patient is compound heterozygous.

Modification Type: Strength

Moderate

- Follow SVI guidance for PM3 (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf).
- Parental testing, or another appropriate molecular method (such as cloning each allele separately followed by sequencing), must have been performed in order to confirm that the variants are in trans if the patient is compound heterozygous.

Modification Type: None

Supporting

- Follow SVI guidance for PM3 (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf).
- Parental testing, or another appropriate molecular method (such as cloning each allele separately followed by sequencing), must have been performed in order to confirm that the variants are in trans if the patient is compound heterozygous.

Modification Type: Strength

PM4

Original ACMG Summary

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Moderate

CCDS VCEP notes: Stop loss variants in GATM have not been reported, as far as we are aware.

GATM specifications: Use this rule “as is” for in frame deletions and insertions of 2 or more amino acids, but downgrade to PM4_Supporting for single amino acid deletions and insertions.

Modification Type: None

Supporting

Downgrade to PM4_Supporting for in frame deletion/insertion of a single amino acid.

Modification Type: Strength

PM5

Original ACMG Summary

Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.
Example: Arg156His is pathogenic; now you observe Arg156Cys.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Moderate

If the pathogenicity of another missense change at the same amino acid residue is unknown, determine its pathogenicity using these specifications in order to determine if this criterion can be used. If the variant is pathogenic, use PM5. If the variant is likely pathogenic, use PM5_Supporting.

Modification Type: None

Supporting

Missense change at an amino acid residue where a different missense change determined to be likely pathogenic has been seen before.

Modification Type: Strength

PM6

Original ACMG Summary

Assumed de novo, but without confirmation of paternity and maternity.

Not Applicable

Comments: CCDS VCEP notes for PS2 and PM6: De novo variants have not been reported in patients with AGAT deficiency, to our knowledge. Furthermore, the observation that a variant in GATM has arisen de novo does not support its causality because AGAT deficiency is an autosomal recessive disorder. The occurrence of de novo variants is more supportive in autosomal dominant and X-linked disorders. Any de novo variants in GATM, should they be observed, will be assessed based on the variant type, functional evidence, and in trans data as described.

PP1

Original ACMG Summary

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.
Note: May be used as stronger evidence with increasing segregation data.

Not Applicable

Comments: CCDS VCEP notes for PP1: Sibships large enough to use meet this criterion are extremely rare. In addition, because GATM is the only gene involved in AGAT-D, ALL patients are expected to be bi-allelic, regardless of whether the pathogenic variants can be, or have been, detected. A variant under assessment may not be the true pathogenic variant but instead in linkage disequilibrium with an unidentified pathogenic variant. For this reason, this criterion does not facilitate assessment of pathogenicity.

PP2

Original ACMG Summary

Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.

Not Applicable

Comments: CCDS VCEP notes for PP2: Does not apply; there are benign and pathogenic missense variants in GATM.

PP3

Original ACMG Summary

Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Moderate

Non-canonical splice site variant predicted to be more deleterious, by SpliceAI and varSEAK, than a previously observed pathogenic variant at the same nucleotide.

Modification Type: None

Supporting

- REVEL score >0.75 for missense variants.
- In frame deletion or insertion predicted deleterious by PROVEAN and MutationTaster.
- Predicted impact on splicing by SpliceAI and varSEAK. GATM specifications:
- For missense changes, those with a REVEL score more than 0.75 will meet PP3.
- For in frame insertions and deletions, use PROVEAN and Mutation Taster. Results must be consistent to count.
- For non-canonical splice site variants (e.g., +3, -3), use SpliceAI ((https://spliceailookup.broadinstitute.org/) and varSEAK (https://varseak.bio/). Results must be consistent to apply this criterion.
- For SpliceAI, any donor loss or acceptor loss with a score >0.5. For varSEAK, any variant with splicing class 4 or 5. Evidence for creation of a cryptic splice site should also be assessed.
- Do not apply this rule for canonical splice site changes meeting PVS1.

Modification Type: None

PP4

Original ACMG Summary

Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology.

Strong

4 or more points based on any combination of the following. Two or more data types are required to meet Strong:
• Low urine guanidinoacetate with or without low or low normal creatine (1 point)
• Low plasma guanidinoacetate with or without low or low normal creatine (2 points)
• Significantly decreased creatine peak in brain magnetic resonance spectroscopy (3 points)
• AGAT enzyme activity <5% of normal (3 points)

* Variant must meet PM2_Supporting for PP4 to apply at any strength.
* For PP4 to be applied at strong, full GATM gene sequencing, including all coding exons and intron/exon boundaries, must have been carried out. If not, consider downgrading.

Modification Type: Disease-specific

Moderate

3 points based on any combination of the following. Two or more data types are recommended to reach moderate:
• Low urine guanidinoacetate with or without low or low normal creatine (1 point)
• Low plasma guanidinoacetate with or without low or low normal creatine (2 points)
• Significantly decreased creatine peak in brain magnetic resonance spectroscopy (3 points)
• AGAT enzyme activity <5% of normal (3 points)

Variant must meet PM2_Supporting for PP4 to apply at any strength.

Modification Type: Strength

Supporting

1-2 points based on:
• Low urine guanidinoacetate with or without low or low normal creatine (1 point)
• Low plasma guanidinoacetate with or without low or low normal creatine (2 points)
Variant must meet PM2_Supporting for PP4 to apply at any strength.

Modification Type: Disease-specific

PP5

Original ACMG Summary

Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant

Interpretation VCEP Review Committee. PubMed : 29543229

BA1

Original ACMG Summary

Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Stand Alone

Allele frequency >0.0005 (0.05%) in gnomAD in any continental population in gnomAD with >2000 alleles.

- Any variant with a frequency >0.0005 (max allelic contribution = 100% and max genetic contribution = 100% based on estimated prevalence of 1 in 3,450,000 (PMID 27233232), and penetrance of 100%) in a continental population with >2000 alleles (European non-Finnish, African, East Asian, South Asian, Latino) (PMID 30311383).
- Use the highest population minor allele frequency (MAF) in any given continental population with >2,000 alleles (European non-Finnish, African, East Asian, South Asian, Latino) (PMID 30311383).

Modification Type: Disease-specific

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

Allele frequency >0.0001 (0.01%) in gnomAD in any continental population in gnomAD with >2000 alleles.

- Any variant with a frequency >0.0001 (max allelic contribution = 25% and max genetic contribution = 100% based on estimated prevalence of 1 in 3,450,000, (PMID 27233232), and penetrance of 100%) in a continental population with >2000 (European non-Finnish, African, East Asian, South Asian, Latino) (PMID 30311383) (see Appendix 3).
- Use the highest population minor allele frequency (MAF) in any given continental population with >2,000 alleles (European non-Finnish, African, East Asian, South Asian, Latino) (PMID 30311383).

Modification Type: Disease-specific

BS2

Original ACMG Summary

Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.

Strong

Observed in the homozygous state in a healthy adult.

Modification Type: Disease-specific

BS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.

Supporting

>30% normal GAA activity when the variant is expressed in a heterologous cell type.

GATM specifications:
In vitro assays in which a variant is expressed in AGAT-deficient cultured cells (e.g. AGAT-deficient fibroblasts) or in-fusion High-Fidelity cloning of GATM transcript and site directed mutagenesis to generate missense variant overexpressed in HeLa cells and measurement of AGAT activity in cells for wild-type and missense variant. Any variant with enzyme activity at or above 30% of normal in DesRoches et al, 2016, PMID 27233232, meets BS3_Supporting.

Modification Type: Disease-specific,Strength

BS4

Original ACMG Summary

Lack of segregation in affected members of a family.
Caveat: The presence of phenocopies for common phenotypes (i.e. cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Not Applicable

Comments: Lack of segregation in a family. Caveat: The presence of

phenocopies for common phenotypes.

BP1

Original ACMG Summary

Missense variant in a gene for which primarily truncating variants are known to cause disease.

Not Applicable

Comments: Missense variant in a gene for which primarily truncating variants are known to cause disease.

BP2

Original ACMG Summary

Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.

Supporting

Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in cis with a pathogenic variant in any inheritance pattern. GATM specifications: Observed in cis with a pathogenic variant (to take AR inheritance into account).

Modification Type: None

BP3

Original ACMG Summary

In frame-deletions/insertions in a repetitive region without a known function.

Not Applicable

Comments: In-frame deletions/insertions in a repetitive region without a known function.

BP4

Original ACMG Summary

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)

Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Supporting

- REVEL score <0.15 for missense variants.
- In frame deletion or insertion predicted benign by PROVEAN and MutationTaster.
- No predicted impact on splicing by SpliceAI and varSEAK. GATM specifications:
- For missense changes, REVEL score <0.15.
- For in frame insertions and deletions, use PROVEAN and Mutation Taster. Results must be consistent to count.
- For non-canonical splice site variants, use SpliceAI (https://spliceailookup.broadinstitute.org/) and varSEAK (https://varseak.bio/) to assess the impact of variants that are not +/-1 or 2 canonical splice site variants. For SpliceAI, this criterion can be applied for scores <0.2, and for varSEAK class 1 and 2. If there is any evidence for possible creation of a cryptic splice site, this criterion should not be applied.

Modification Type: None

BP5

Original ACMG Summary

Variant found in a case with an alternate molecular basis for disease.

Not Applicable

Comments: Variant found in a case with an alternate molecular basis for disease. CCDS VCEP notes for BP5: An individual could be a carrier of a pathogenic variant in GATM and have another disorder

BP6

Original ACMG Summary

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BP7

Original ACMG

Summary

A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Supporting

A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Modification None
Type:

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1, PM3_Very Strong)* **AND ≥ 1 Strong** *(PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)*

1 Very Strong *(PVS1, PM3_Very Strong)* **AND ≥ 2 Moderate** *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)*

1 Very Strong *(PVS1, PM3_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)* **AND 1 Supporting** *(PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)*

1 Very Strong *(PVS1, PM3_Very Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)*

≥ 2 Strong *(PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)*

1 Strong *(PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)* **AND ≥ 3 Moderate** *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)*

Likely Pathogenic

1 Very Strong *(PVS1, PM3_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)*

1 Very Strong *(PVS1, PM3_Very Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)*

≥ 3 Moderate *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)*

2 Moderate *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)*

1 Moderate *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)*

Benign

≥ 2 Strong *(BS1, BS2)*

1 Stand Alone *(BA1)*

Likely Benign

1 Strong *(BS1, BS2)* **AND 1 Supporting** *(BS3_Supporting, BP2, BP4, BP7)*

≥ 2 Supporting *(BS3_Supporting, BP2, BP4, BP7)*